

PMC9 / Tag, T: D.V, DV.

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purpose: Amplification of PMC9 with different amounts of Tag - titration.
 Deep vent
 Tag + Deep vent
 Constant
 different con - titration
 all samples discarded

using 40 primers, 1 μ M
 200 μ M dNTP
 1 μ M primer do ϕ 2728 & 2729 (100 μ M)
 100 μ g template diluted 100 μ g / λ \rightarrow 1 μ g / λ
 Deep vent buffer

Titration: a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 U
 b. Tag: Deep vent: 1:0, T1: 0.001, 1:0.05, 1:1.0, 1:1.05
 1:0.1, 1:2, 1:0.5, 1:1, 1:2
 c. Deep vent 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5

prepared a master mix w/o enzyme. Enzyme added separately.

Did just one of each.
 45 μ l + 5 μ l (enzyme + H₂O)
 H₂O 1167
 10 x buffer 150
 primer 1 15
 2 15

Tag: 5 U / λ diluted to 1 U / λ in 1x buffer. T. template 3 μ l
 Enzyme H₂O
 0 = - 5 D.V 2 U / λ \rightarrow 1 U / λ \rightarrow 0.1 U / λ 45 μ l
 1.5 = 0.5 4.5 0.001 U / λ
 1 = 1 4 0.025 2.5 } 0.01
 1.5 = 1.5 3.5 0.05 5 }
 2 = 2 3 0.1 1 } 0.1
 2.5 = 2.5 2.5 0.5 5 }
 5 = 5.0 - 1.0 0.5 } 2 U / λ
 2.0 = 4.0 5.0 = 2.5 μ l To Page No. _____

Used & Understood by me,

[Signature]

Date

11/18/94

Invented by

Recorded by

K. Sitarman

Date

11/18/94

<u>T</u>	<u>D.V</u>	<u>Tag</u>	<u>W/x</u>	<u>D.V</u>	<u>calculated in</u> <u>1x buffer</u>	<u>Tube #</u>
Unit 1	0	1	μl			16
	0.001			1	> 0.001 W/x	17
	0.005			5		18
	0.01			1	> 0.01 W/x	19
	0.05			5		20
	0.1			1	> 0.1 W/x	21
	0.5			5		22
	1.0			15	> 2.0 W/x	23
	2.0			1		24

= Thermocycled at $94^{\circ}, 5'$

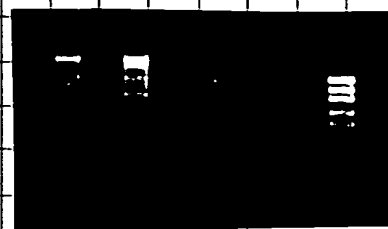
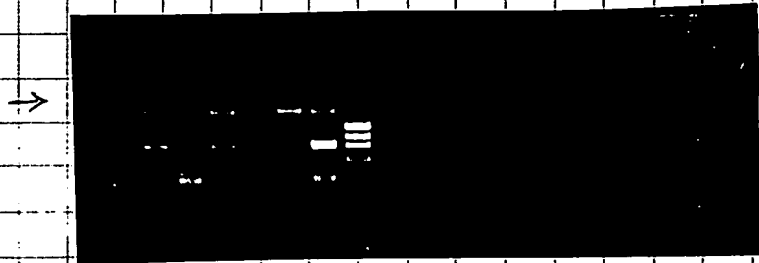
30 (94°, 30', 56", 45 sec, 72', 3")

- after 6 cycles noticed dNTP was n: added (1) ~ 20 added 1 μ l of 10 mM dNTP (200 μ M / 50 μ l 2x) individually each time & started again the cycling!

maybe have to repeat again

Tag alone Deep vent' alone

Tag : Deep vent



No- Contamination? ☒ (w/ enzyme)

- so much misprinting

Repeat α in dyspepsia, use Lambda / 14 and 15 marker

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Witnessed & Understood by me,

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Inv nted by

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Recorded by

11/21/84